TECHNICAL REPORT
68-6-FL

GROWTH OF PLANT CELL CULTURES I. ISOLATION OF CULTURES, SELECTION OF MEDIA, & EFFECTS OF FREQUENCY OF TRANSFER

By

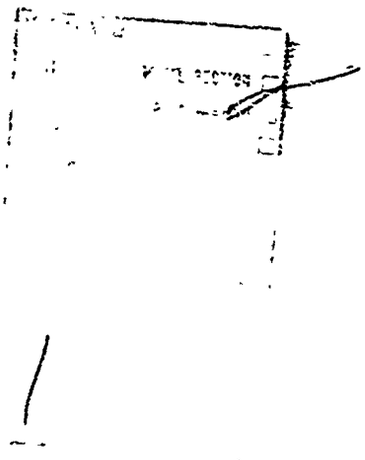
Mary Mandels
Anne Maguire
and
Hamed M. El-Bisi

July 1967

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760

Food Division
FL-58

66



This document has been approved for public release and sale; its distribution is unlimited.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

Destroy this report when no longer needed. Do not return to the originator.

AD_____

TECHNICAL REPORT
68-6-FL

GROWTH OF PLANT CELL CULTURES

I. Isolation of Cultures, Selection of Media, and Effects of Frequency of Transfer

by

Mary Mandels, Anne Maguire and Hamed M. El-Bisi
Microbiology Division

Project reference:
1J014501A71C

Series: FL-58

July 1967

Food Laboratory
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts 01760

FOREWORD

This report represents the initial phase of the work on culture of cells of higher plants undertaken at Natick under the unconventional food program in the Microbiology Division of the Food Laboratory. The objective of this study is to determine whether it would be feasible to use plant cell cultures as a source of human food. The objective of this phase was to isolate and establish suitable cell lines and to investigate their growth kinetics and productivity on simple media preliminary to initiating mass culture studies.

FERDINAND P. MEHRLICH, Ph.D.
Director
Food Laboratory

APPROVED:

DALE H. SIELING, Ph.D.
Scientific Director

W. M. MANTZ
Brigadier General,
Commanding

TABLE OF CONTENTS

	Page No.
List of Tables	iv
List of Figures	v
Abstract	vi
Introduction	1
Methods	5
Results	14
Part A. Growth of plant cells on solid media	14
Experiment 1. Effect of medium	14
Experiment 2. Effect of number and frequency of transfers	16
Part B. Growth of plant cells in suspension	17
Experiment 1. Effect of medium	17
Experiment 2. Effect of number and frequency of transfers	27
Experiment 3. Effect of thickening agents	47
Discussion	49
References	54

LIST OF TABLES

Table		Page No.
1	White's Medium for Growth of Plant Tissue Cultures (26) . . .	6
2	Murashige and Skoog Medium for Growth of Plant Tissue Cultures (11)	7
3	Hildebrandt's C Medium for Growth of Plant Tissue Cultures (7)	8
4	Becker (Torrey M-6) Medium for Growth of Cambium Tissue (1)	9
5	Tsuchiya Medium for Growth of Orchid Meristems (27)	10
6	Plant Cell Cultures at Natick	11
7	Growth of Plant Cell Cultures on Solid Media at 28°C (27-54 days)	15
8	Effect of Frequency of Transfer on Growth of Plant Cell Cultures on Solid Murashige Medium in the Dark at 28°C . . .	18
9	Growth of Plant Cells in Suspension Culture at 28°C (23-69 days)	25
10	Effect of Frequency of Transfer on Growth of Suspension Cultures of Carrot Cells. Dark 28°C	33
11	Effect of Frequency of Transfer on Growth of Suspension Cultures of Lettuce Cells. Dark 28°C	34
12	Effect of Frequency of Transfer on Growth of Suspension Cultures of Bean Cells. Dark 28°C	35
13	Effect of Thickening Agents on Growth of Plant Cell Suspension Cultures Murashige Medium + 1g phytone per L - Dark 28°C. 28 days.	48
14	Yields of Plant Cell Material in Suspension Culture	51
15	Plant Yields in Total Dry Organic (Ash Free) Matter in Metric Tons per Hectare per Year. After Westlake 1963 (25)	52

LIST OF FIGURES

Figure		Page No.
1	Average Growth of Plant Cells on Solid Murashige Medium over a 36 Week Period. Dark 28°C	19
2	Effect of Frequency of Transfer on Growth of Plant Cells on Solid Murashige Medium over a 36 Week Period. Dark 28°C. Growth Increment and Productivity Figures are Averages for the 36 Week Period	21
3	Effect of Frequency of Transfer on the Cumulative Growth Increment of Plant Cells on Solid Murashige Medium over a 36 Week Period. Dark 28°C	23
4	Effect of Age of Inoculum on Growth of Plant Cell Suspension Cultures on Murashige Medium. Dark 28°C	29
5	Effect of Medium on Growth of Plant Cell Suspension Cultures. Dark 28°C	31
6	Effect of Frequency of Transfer on Growth Rate of Suspension Cultures of Plant Cells over a 26 Week Period. Dark 28°C	37
7	Effect of Frequency of Transfer on Cumulative Growth Increment of Carrot Cells in Suspension Culture on Murashige Medium. Dark 28°C	39
8	Effect of Frequency of Transfer on Cumulative Growth Increment of Bean Cells in Suspension Culture on Murashige Medium. Dark 28°C	41
9	Effect of Frequency of Transfer on Cumulative Growth Increment of Bean Cells in Suspension Culture on Murashige Medium Supplemented with One Gram of Phytone Per Liter. Dark 28°C	43
10	Effect of Frequency of Transfer on Productivity of Dry Matter in Suspension Cultures of Plant Cells over a 28 Week Period. Dark 28°C	45

ABSTRACT

Callus cultures have been isolated from a number of edible plants and maintained on simple defined media for extended periods. Growth rates are slow in comparison to other microbial systems, and increase tends to be linear. Static cultures on solid media double in 5-10 days, yield up to 0.26 mg dry weight per ml per day, and attain a maxiumum weight of about 12 mg dry weight per ml. Suspension cultures double in 2-5 days, yield up to 1.1 mg dry weight per ml per day, and attain a maximum weight of about 23 mg dry weight per ml. These growth rates are of the same order of magnitude as those for higher plants growing conventionally. Considerable improvements in these growth rates will be required before use of plant cell cultures as food can be realized economically.

Introduction

The culture of undifferentiated plant cells began in the 1930's with the pioneer studies of White, Robbins, Gautheret and others (26) Progress was slow until 1942 when Van Overbeek and Blakeslee (28) introduced the use of liquid endosperm of the coconut for the culture of plant embryos, and it was discovered that coconut milk was an excellent source of organic nutrients and growth factors for many plant cells. Today cells isolated from all parts of the plant, and representing a variety of plant families are being successfully cultured (6). As knowledge of plant hormones and growth factors has developed it has become possible to culture many plant cells on fully defined media, either as masses on solid media, or in suspension in liquid media (11, 18). Such cultures in many ways resemble microorganisms, and can be grown and handled in similar fashion. Among the products identified in such cultures are: alkaloids including nicotine, atropine, hyascine, tomatine, and candicine; amino acids; proteins; enzymes including amylase, invertase, catalase, peroxidase, indole acetic acid oxidase, polyphenol oxidase, protease, and pectin methylesterase; carbohydrates including starch; glycosides; organic acids; pigments including chlorophyll, carotenoids, xanthophyll; flavonoids, and anthocyanins; phenolics; tannins; lignins; saponins; steroids; terpenoids; antibiotics against Staphlococci and Mycobacteria; and growth regulators (gibberellins) (4).

In the past few years mass culture of plant cells has been achieved in a number of laboratories. Nickell and Tulecke at Pfizer (12, 13, 14, 22) grew plant cells in suspension and scaled up production to pilot plant scale. They achieved yields of 3 g (wet weight) per liter per day for carboy cultures of Gingko, Ilex, and Lolium cells, and 10 g (wet weight) per liter per day in a 134 liter pilot plant fermentor. The last was a two day run using a heavy inoculum. Pfizer had hoped to grow, in vitro, tissues of plants that produce valuable pharmaceuticals or other products. Because of the 10 - 20% coconut milk incorporated in the medium and the slow growth rate, the economics did not appear favorable. Furthermore, it has been repeatedly shown (19, 24) that marked biochemical differences may exist between the same tissue in culture and in the intact plant. Unfortunately compounds of pharmaceutical interest tend to occur in lower concentrations in the cultured tissue. Pfizer has dropped its plant tissue culture project.

Staba at the University of Nebraska (15, 16, 23) has also grown mass cultures of plant tissues hoping to produce pharmaceuticals. In a dual carboy system he achieved a yield of 4.7 g (wet weight) of spearmint tissue per liter per day in an 8-day run. In Erlenmeyer flasks he obtained a yield of 6.3 g (wet weight, 0.4 g dry weight) per liter per day in 15 days growth.

Anna Byrne at the Quartermaster Food & Container Institute in Chicago (2, 3) has studied the mass culture of plant cells as an unconventional food source. Her greatest success was with carrot tissue. Using a 15% coconut milk medium, she achieved yields of up to 6.1 g (wet weight) per liter per day in a 6 liter carboy. In a semi-continuous system yields were up to 1.9 g dry weight per liter of culture per day. Proximate analyses of the carrot tissue varied widely depending on media and growth conditions, but the range was from 13 - 36% protein, 6 - 42% fat, 2 - 9% ash, and 15 - 28% crude fiber.

Tulecke has continued his studies of mass culture of plant cells at Boyce Thompson Institute with Air Force support (20, 21). He now uses no coconut milk, but grows his tissue on fully defined (Murashige) medium in a semi-continuous 8 liter "phytostat." At intervals of one or two days, about a liter of culture was harvested and an equal volume of fresh medium added. In 7 runs totaling 222 days, he harvested 163 liters of rose culture with an average fresh weight of 112 g (4.6 g dry weight) per liter; or a production of 0.42 g dry weight per liter of culture per day. The generation time was 7 to 8 days. The harvested tissue had 3.4% dry weight, 16% of this was protein. Less dense cultures had up to 19% protein, slower growing cultures had less protein (as little as 7%). Test diets were prepared for weanling mice in which

the protein was supplied by either rose tissue or casein. Mice fed over a 20 day period did poorly on either diet as compared to the standard laboratory Wayne Lab-blox feed. It appears that the test diets were not well formulated.

For use in a bioregenerative system, it would be desirable to have a photosynthetic plant tissue culture. So far, this has not been achieved. Hildebrandt at Wisconsin has selected tissues of moderately high chlorophyll content from a number of edible plants, but there is no apparent difference in the nutritional requirements of these from strains lacking chlorophyll (8). Cultured chlorophyllous tissue has been shown to fix carbon dioxide in light (9, 17) but in contrast to tissues in intact plants, the fixed carbon does not appear in sucrose. In general there appears to be an inverse relation between chlorophyll content and growth rate (4, 16, 17).

Current studies in our laboratory are directed towards:

(a) better understanding of growth kinetics and, hence, development of useful growth indices,

(b) further understanding of growth and nutritional requirements in an attempt to develop simple economic media,

(c) selecting for, inducing, and enhancing chlorophyllous growth in an attempt to attain photoautotrophic conditions and

(d) defining parameters for and eventually attaining sound economic continuous mass-culture systems.

Methods

Media used in this study are shown in Tables 1-5.

Cultures used in this study (Table 6) are all callus cultures isolated by us; most from food plants. Seeds were sterilized in 5% calcium hypochlorite and planted without rinsing on White's medium with agar, then grown for one to four weeks at 28°C in a New Brunswick Incubator (G27) under continuous white fluorescent light (840 foot candles). Seedlings were cut up aseptically and small pieces were transferred to agar slants of White's medium or Murashige medium with or without supplements; 10% coconut milk; or 0.1 - 1.0 g/l of phytone (BBL), tryptone (Difco), yeast extract, corn steep, proflo (defatted cotton seed flour), or casein hydrolysate; or 0.1 - 5.0 mg/l of α naphthaleneacetic acid, 2.4 dichlorophenoxyacetic acid, or calcium pantothenate. Plant pieces were incubated for several weeks in the light at 28°C or in the dark at 24-28°C. When callus tissue formed at cut surfaces it was transferred to fresh medium (date of isolation). Carrot cell cultures were isolated from mature roots rinsed with alcohol and a cylinder of phloem tissue removed with a sterile cork borer. This tissue was cut up and handled like the seedling pieces. Isolations of callus tissues were usually made 4-6 weeks after placing cut up tissue on agar. After that cultures were transferred at intervals of 4-6 weeks and incubated in the dark or light at 28°C.

Table 1. White's Medium for Growth of Plant Tissue Cultures (26).

Component	mg/Liter
$MgSO_4$	360.
$Ca(NO_3)_2 \cdot 4H_2O$	200.
$Na_2SO_4 \cdot 10H_2O$	200.
KCl	80.
$NaH_2PO_4 \cdot H_2O$	16.5
$MnSO_4 \cdot 7H_2O$	4.5
$ZnSO_4 \cdot 7H_2O$	1.5
H_3BO_3	1.5
KI	0.75
Ferric tartrate	40.
Glycine	3.0
Nicotinic acid	0.5
Thiamine	0.1
Pyridoxine	0.1
Sucrose	20,000.

pH 5.5

Optional additives

Agar 6 g/L

Coconut Milk 100 ml/L

Table 2. Murashige and Skoog Medium for Growth of Plant Tissue Cultures (11).

Component	mg/Liter
KNO_3	1900.
NH_4NO_3	1650.
$CaCl_2 \cdot 2H_2O$	440.
$MgSO_4 \cdot 7H_2O$	370.
KH_2PO_4	170.
$MnSO_4 \cdot 4H_2O$	22.3
$ZnSO_4 \cdot 4H_2O$	8.6
H_3BO_3	6.3
KI	0.83
Molybdic acid	0.25
$CuSO_4 \cdot 5H_2O$	0.25
$CoCl_2 \cdot 6H_2O$	0.25
Sodium EDTA	37.3
$FeSO_4 \quad 7H_2O$	27.8
Inositol	100.
Glycine	2.
Nicotinic acid	0.5
Pyridoxine	0.5
Kinetin (6-furfuryl aminopurine)	0.32
Thiamine	0.1
2.4 dichlorophenoxyacetic acid (2,4D)	0.05
Sucrose	30,000.

pH 5.8

Optional additives

Agar 6g/L

Phytone 1g/L

α napthaleneacetic acid (NAA) 0.1 mg/L (omit 2,4D)

Table 5. Hildebrandt's C Medium for Growth of Plant Tissue Cultures (7).

Component	mg/Liter
Na_2SO_4	800.0
$Ca(NO_3)_2 \cdot 4 H_2O$	400.0
$Mg SO_4 \cdot 7 H_2O$	180.0
$K NO_3$	80.0
KCl	65.0
NaH_2PO4	33.0
$Zn SO_4 \cdot 7 H_2O$	6.0
$Mn SO_4 \cdot 4 H_2O$	4.5
KI	3.0
$H_3 BO_3$	0.375
Ferric tartrate	40.0
Glycine	3.0
Ca pantothenate	2.5
Thiamine	0.1
α naphthalene acetic acid (NAA)*	0.1
Sucrose	20,000.0

pH 5.5

Optional additives

Agar 6g/L

Coconut Milk 10%

2,4D 6mg/L (*Omit NAA = D medium)

Table 4. Becker (Torrey M-6) Medium for Growth of Cambium Tissue (1).

Component	Mg/Liter
$Ca\ NO_3$	242.
$K\ NO_3$	85.
KCl	61.
$Mg\ SO_4 \cdot 7\ H_2O$	42.
$K\ H_2\ PO_4$	20.
$Fe\ Cl_2$	25.
Yeast Extract	1,000.
2,4D	2.*
Sucrose	40,000.

pH 5.5

Optional additives

Agar 6 g/L

*9×10^{-6}M

Table 5. Tsuchiya Medium for Growth of Orchid Meristems (27).

Component	mg/Liter
$K NO_3$	525.
$(NH_4)_2 SO_4$	500.
$K H_2 PO_4$	250.
$Mg SO_4$	250.
$Ca H PO_4$	200.
Ferric tartrate	30.
Tryptone	2,000.
Sucrose	20,000.

pH 5.5

Optional additives

Agar 6 g/L

Table 6. Plant Cell Cultures at Natick

Culture No.	Isolation Date	Plant	Variety	Part
1	6/15/64	Pepper *Capsicum sp*	Hot Vietnamese	Stem
2	6/15/64	Carrot *Daucus carota*	Unknown**	Root
3*	6/15/64	Squash *Cucurbita pepo*	Cocozella	Stem
4*	6/15/64	Radish *Raphanus sativus*	Champion	Stem
5*	6/15/64	Radish	Champion	Stem
6*	6/15/64	Radish	Champion	Stem
7	6/29/64	Lettuce *Lactuca sativa*	Grand Rapids	Leaf
8	7/ 7/64	Bean *Phaseolus vulgaris*	Tendercrop	Leaf
9*	7/16/64	Cucumber *Cucumis sativa*	China	Stem
10	7/16/64	Lettuce	Grand Rapids	Leaf
11	7/20/64	Carrot	Unknown**	Root
12	7/20/64	Bean	Tendercrop	Stem
13	7/20/64	Lettuce	Grand Rapids	Leaf
14*	7/20/64	Cucumber	China	Stem
15*	7/28/64	Bean	Tendercrop	Cotyledon
16	7/28/64	Cucumber	China	Stem

Table 6. (continued)

Culture No	Isolation Date	Plant	Variety	Part
17*	7/28/64	Bean	Tendercrop	Stem
18	7/28/64	Carrot	Unknown**	Root
19*	7/28/64	Cucumber	China	Stem
20*	7/28/64	Bean	Tendercrop	Stem
22*	7/28/64	Marigold *Tagetes erecta*	Marigolds on parade	Stem
23	7/29/64	Lettuce	Grand Rapids	Leaf
24	7/29/64	Lettuce	Grand Rapids	Root
25*	7/29/64	Marigold	Marigolds on parade	Stem
26	7/29/64	Marigold	Marigolds on parade	Stem
27	7/29/64	Marigold	Marigolds on parade	Stem
28*	7/29/64	Marigold	Marigolds on parade	Stem
29*	8/ 3/64	Bean	Tendercrop	Stem

*Culture no longer on hand

**Cultivated - purchased in market

Liquid suspension cultures were initiated by inoculating a piece of callus tissue from an agar slant into a shake flask. Erlenmeyer flasks were used with 25 ml medium per 125 ml flask, 50 ml medium per 250 ml flask or 100 ml medium per 300 or 500 ml flask. Cultures were grown on rotary shakers (variable speeds) in the dark or in continuous fluorescent light of about 200 foot candles, or on a reciprocating shaker (90 strokes per minute) in the dark, at 28°C.

Growth was measured as dry weight in mg per ml. Static cultures were scraped from the agar washed in water and dried at 80°C. Suspension cultures were harvested in toto, or if well dispersed, two 10 ml samples were taken with a large opening pipette, filtered by suction through tared Schleicher and Shuell 5.5 cm No 596 filter paper circles, washed in water, and dried at 80°C. Growth rate is expressed as Growth Increments (GI) = $\left(\frac{\text{final wt}}{\text{inoculum}} - 1\right)$ per unit time. Productivity (P) is expressed as mg per ml per day = $\left(\frac{\text{final wt - inoculum}}{\text{days growth}}\right)$.

Results

Part A. Growth of plant cells on solid media.

Experiment 1. Effect of medium.

This experiment was designed to select the best cultures for further experimentation and to select a suitable basic medium for their cultivation. Media used were White's plus 10% coconut milk, Murashige, or Hildebrandt's C plus 10% coconut milk. Preliminary tests had shown that growth of callus tissue was very slow on White's or Hildebrandt's media in the absence of coconut milk. 10 ml agar slants were inoculated with about 10 mg dry weight of tissue (1 mg per ml) and incubated at 28°C in light or dark for 27-54 days. The values shown (Table 7) represent the best weights obtained in three tests.

Pepper tissue is firm, white, and slow-growing with a crisp texture and a faint pepper flavor. It does not turn green, and does not differentiate organs. Carrot tissue is friable, fast growing, white or yellow in the dark, green in the light. It frequently produces adventitious plants even on media without coconut milk, and new growth types frequently appear in these cultures. The flavor is bland. Radish cultures are slow growing, white and compact. They frequently produce roots. Lettuce cultures are fast growing and short lived. The tissue is friable, yellow or greenish in the dark, very green in light, and produces a soluable yellow pigment

Table 7. Growth of Plant Cell Cultures on Solid Media at 28°C (27-54 days).

Culture (No)	Dry weight (mg/ml) using (medium) Murashige	White CM	Hildebrandt's CM
Pepper			
1	6.5	4.0	-
Carrot			
2	7.4	10.2	8.6
11	6.8	9.4	8.5
18	8.1	9.3	7.9
Radish			
4	3.7	-	5.7
6	1.9	1.0	-
Lettuce			
7	10.1	10.5	9.3
10	9.5	8.9	9.8
13	10.5	12.2	9.3
23	9.0	8.0	7.9
24	9.4	9.0	9.5
Bean			
8	11.6	7.6	8.8
12	8.7	7.9	7.1
Cucumber			
9	2.7	2.2	4.4
14	3.0	-	0.3
16	1.1	0.7	2.7
19	2.0	0.8	2.8
Marigold			
25	12.3	8.6	7.0
26	10.5	7.8	5.3
27	8.4	4.3	8.0
28	10.4	8.1	5.7

CM = 10% coconut milk
- = No test

that diffuses into the agar. Cultures in the light differentiate many small leaves and plantlets. Such cultures are crisp, taste like garden lettuce, and should make a good salad. Bean cultures are fast growing, white when young, but turning brown as the culture ages. These cultures do not turn green in the light and have never differentiated leaves, roots, or plants. Young cultures taste like raw bean. Old cultures are bitter. Cucumber cultures are slow growing, white and friable in the dark, green in the light. They have a refreshing odor of sliced cucumbers. Marigold cultures are fast growing, white at first, but rapidly turning black. They do not turn green in the light. They produce an abundance of adventitious roots, but no leaves or plants.

Since growth of these cultures on the fully defined Murashige medium was as good or better than on the media with coconut milk, Murashige was selected as the basic medium for maintenance of cultures and further experimentation.

Experiment 2. Effect of number and frequency of transfers.

This experiment was designed to investigate growth rates of carrot, lettuce and bean cells on Murashige medium, and the effect on these growth rates of the frequency of transfer. All cultures were grown in the dark at 28°C on 10 ml agar slants. The inoculum was cut into uniform pieces, each slant was inoculated with a piece, five pieces were

dried and weighed to obtain inoculum weight. Final weights represent an average of 2 or 3 cultures (Table 8, Figures 1, 2, 3).

Bean cultures reached maximum weight in about 3 weeks. This was the only culture that grew rapidly enough to be maintained with a weekly transfer (25-50% inoculum) and this series had the most rapid growth rate (GI = 1.5 per week). Bean cultures transferred at 2 or 3 week intervals showed maximum productivity of 0.26 mg per ml per day. Lettuce cultures showed a greater lag with most rapid growth from 2-6 weeks at which time maximum weight was reached. Cultures declined in weight after 6 weeks. Carrot calls had the longest lag and did not begin growing until 3 weeks - these cultures also reached maximum weight at 6 weeks followed by a decline. It is interesting that these cultures that decline in old age also show a long lag on transfer.

Part B. Growth of plant cells in suspension.

Experiment 1. Effect of medium.

As in Part A, this experiment was designed to select the best cultures for future experimentation and to select the best basic medium for their cultivation. Media used were White's plus 10% coconut milk, Murashige, Hildebrandts' C, Becker and Tsuchiya. Initial transfers grew slowly and

Table 8. Effect of Frequency of Transfer on Growth of Plant Cell Cultures on Solid Murashige Medium in the Dark at 28°C

Culture	Transfer Interval Weeks	No Transfers	No Weeks	Ave. Inoc. mg/ml	Ave. Final mg/ml	Ave. GI per week	Ave. P mg/ml/day
2 carrot	1	3*	3	0.40	0.40	0.2	0
	2	18	36	0.86	2.00	0.8	0.08
	3	11**	33	1.00	2.80	0.7	0.08
	4	9	36	1.50	6.50	1.1	0.18
	6	6	36	1.90	11.00	0.7	0.18
	8	3*	24	1.70	5.40	0.4	0.08
7 lettuce	1	5*	5	0.34	0.50	0.8	0.02
	2	18	36	0.98	3.00	1.1	0.14
	3	12	36	1.20	5.50	1.0	0.21
	4	9	36	1.70	7.70	1.0	0.21
	6	6	36	1.80	9.80	0.9	0.19
	8	3*	24	1.60	6.00	0.4	0.08
12 bean	1	33*	33	1.20	2.70	1.5	0.21
	2	18	36	1.50	4.10	1.3	0.26
	3	12	36	1.90	7.30	1.4	0.26
	4	9	36	1.70	7.50	1.0	0.21
	6	6	36	1.70	7.80	0.7	0.14
	8	5***	38	1.80	7.00	0.6	0.07

*Discontinued due to slow growth
**Discontinued due to contamination
***Last transfer grew only 6 weeks

$$GI = \text{Growth Increment} = \frac{\text{Final}}{\text{Inoc}} - 1 \text{ (dry wt)}$$

$$P = \text{Production} = \frac{\text{Final} - \text{Inoc}}{\text{No days}} \text{ (dry wt)}$$

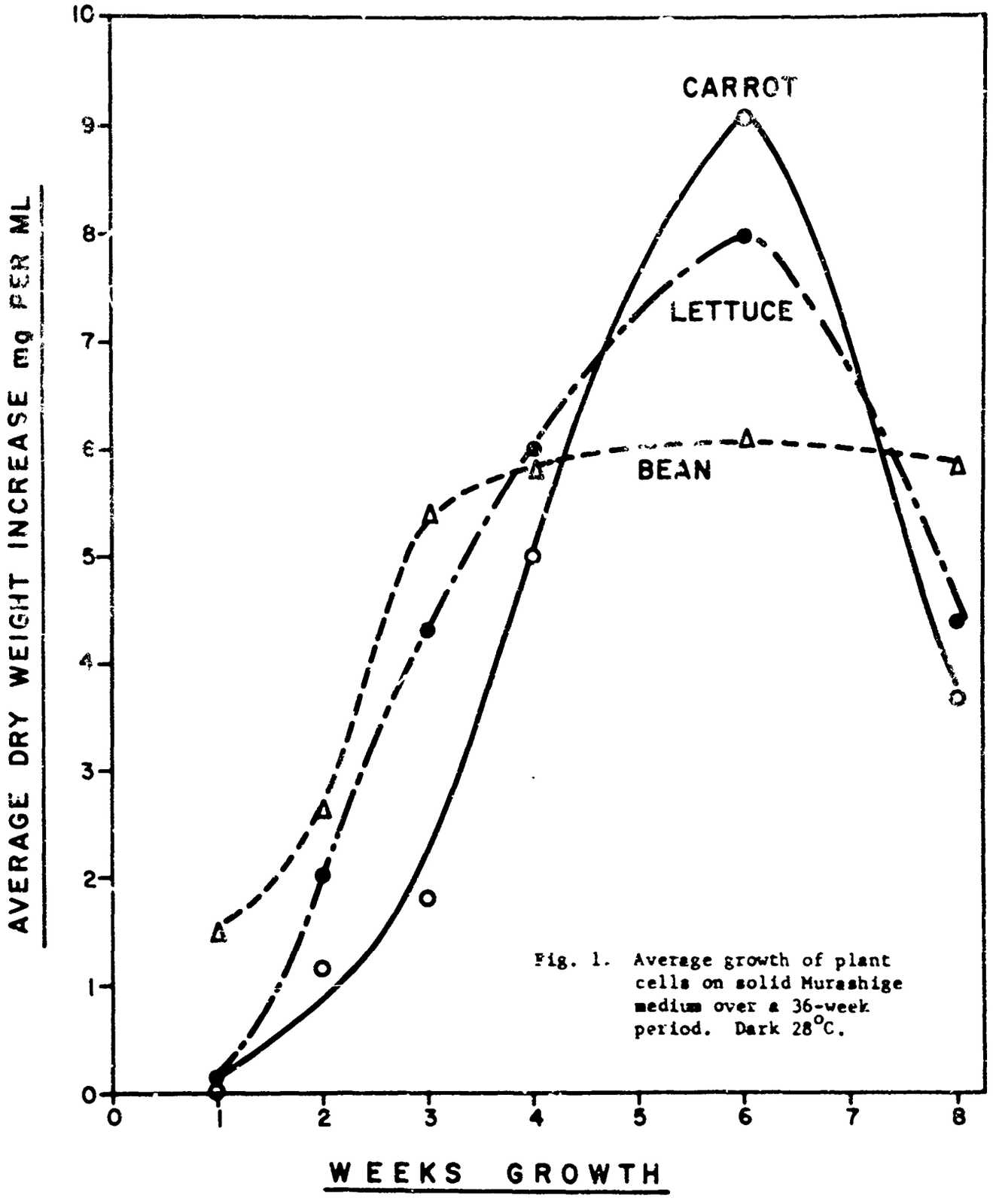
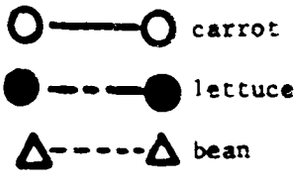


Fig. 1. Average growth of plant cells on solid Murashige medium over a 36-week period. Dark 28°C.

Fig. 2. Effect of frequency of transfer on growth of plant cells on solid Murashige medium over a 36 week period. Dark 28°C. Growth increment and productivity figures are averages for the 36 week period.



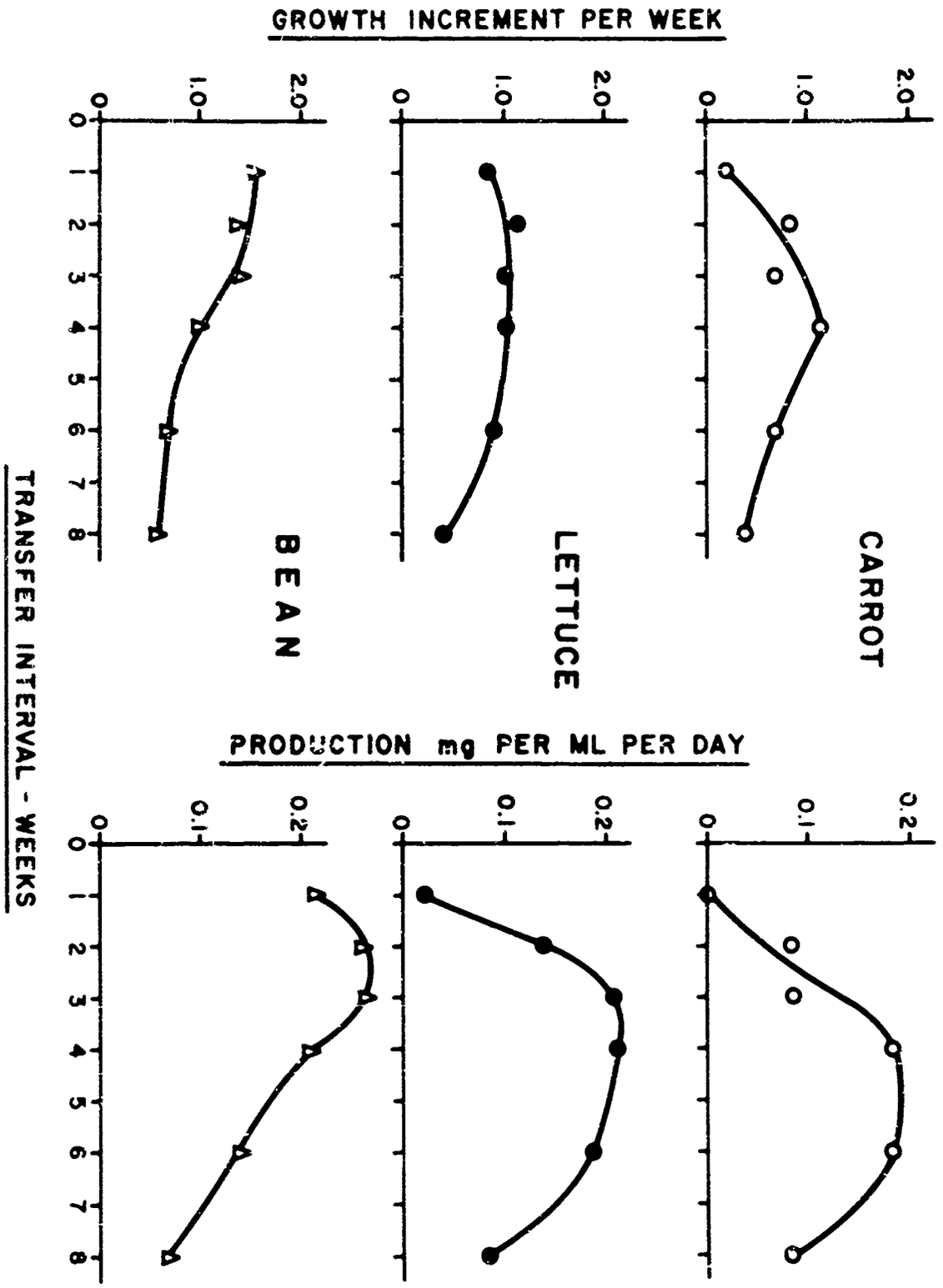
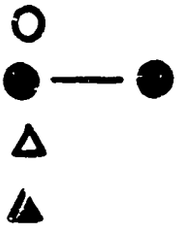

GROWTH INCREMENT PER WEEK
0
1.0
2.0
CARROT
LETTUCE
BEAN
PRODUCTION mg PER ML PER DAY
0
0.1
0.2
0 1 2 3 4 5 6 7 8
TRANSFER INTERVAL - WEEKS

Figure 2

Fig. 3. Effect of frequency of transfer on the cumulative growth increment of plant cells on solid Murashige medium over a 36 week period. Dark 28°C.



transfer every week

transfer every two weeks

transfer every four weeks

transfer every eight weeks

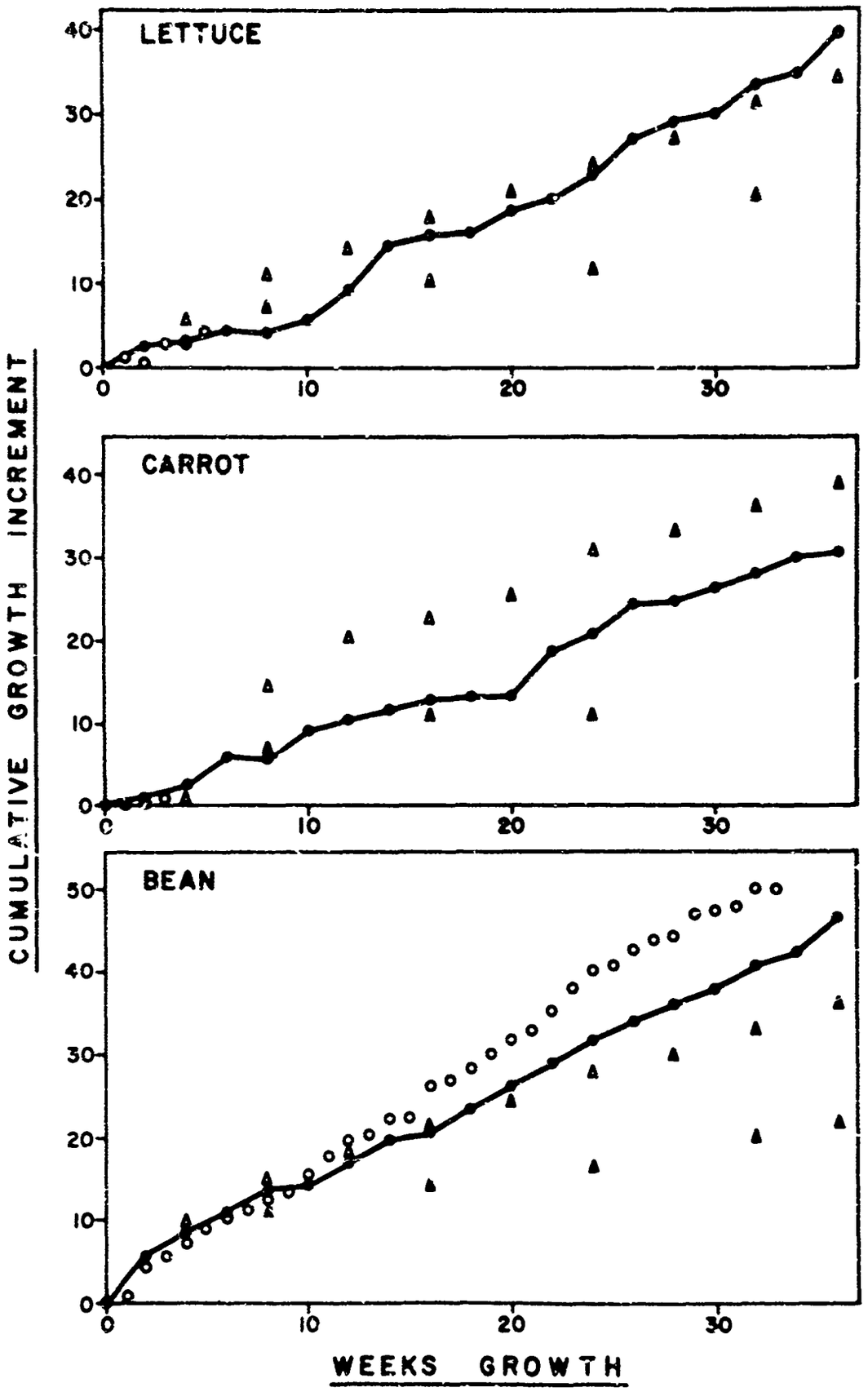

LETTUCE
CARROT
BEAN
CUMULATIVE GROWTH INCREMENT
WEEKS GROWTH
0
10
20
30
40
50

Figure 3

often as large masses. However, some cells usually sloughed off and grew free or as small masses in the medium. By making successive transfers with a large opening pipette a number of cultures were obtained that grew in good suspension. All cultures show a strong tendency to vary in their growth characteristics with successive transfer. Growth potential depends as much on a good inoculum as on a suitable medium. Inocula were around 0.5 mg dry weight per ml, either solid or liquid (10% by volume). Cultures were grown in the light or dark at 28°C for 23-69 days, usually 24-40 days. The values shown (Table 9) represent the best weights obtained in two to ten tests. They are not corrected for a 5-10% decrease in volume due to evaporation of the medium during growth.

The pepper and cucumber have not been obtained as good suspension cultures, but grow only as large masses. The values of around 2% dry weight obtained for carrot, bean, and lettuce cells represent 60-70% conversion to plant cell matter of the 3% sucrose supplied in the medium. Such cultures are very thick and do not settle on standing.

The condition of the inoculum has a marked effect on growth of plant cell cultures. For suspension cultures the best inoculum is often obtained from cultures three or four weeks old (Figure 4). In these cultures there is a lag of one or two weeks followed by a period of rapid growth which is aritnmetic rather than exponential. This suggests that most of the cells in the culture do not divide. Microscropic examination of older cultures

Table 9. Growth of Plant Cells in Suspension Culture at 28°C (23-69 days).

Culture (No)	Murashige	White CM	Hildebrandt CM	Isuchiya	Becker
	Dry weight (mg/ml) in (medium)				
Pepper					
1	4.4	0.4	2.8	-	-
Carrot					
2	21.0	15.0	10.0	13.0	6.7
11	3.9	-	3.0	8.7	3.7
18	15.0	5.8	8.2	15.0	2.9
Radish					
4	2.2	5.3	-	-	-
5	0.4	-	-	-	-
6	0.5	-	-	-	-
Lettuce					
7	10.0	9.0	6.5	3.5	5.2
10	3.0	-	2.8	1.9	3.3
13	18.0	7.2	8.6	2.0	12.0
23	0.2	-	6.9	-	-
24	14.0	-	9.4	2.4	7.2
Bean					
8	23.0	12.0	6.7	-	-
12	11.0	10.0	6.6	8.9	-
15	-	-	0.3	-	-
17	-	-	0.2	-	-
20	-	-	5.0	-	-
Cucumber					
9	1.9	1.6	2.0	0.7	0.7
14	-	-	0.8	-	-
16	0.6	1.0	1.6	-	-
19	-	-	1.2	-	-

Table 9. (continued)

Culture (No)	Murashige	White CM	Hildebrandt CM	Tsuch ya	Becker
	Dry weight (mg/ml) in (medium)				
Marigold					
22	-	-	3.7	-	-
25	3.0	-	4.4	4.2	3.2
26	5.8	-	4.4	-	-
27	-	-	3.5	-	-
28	8.1	-	2.0	-	-

CM = 10% v/v coconut milk
- = No Test

reveals many cell fragments. Doubling times, depending on which part of the curve is examined, range from 3 to 12 days. In other curves (not shown) doubling times of 1 or 2 days have been observed.

Variations in growth on different media are shown (Figure 5). These cultures were inoculated from suspension cultures on Murashige medium, 2 or 3 weeks old. Phytone often causes a marked stimulation of growth on Murashige medium, especially for young cultures.

As a result of these studies, Murashige medium was selected as the basic medium for all cultures, since it supports growth as well or better than other media, and in addition is fully defined.

Experiment 2. Effect of number and frequency of transfers.

This experiment was designed to study the effect of frequency of transfer on the growth rate of suspension cultures of plant cells. Our hope was that frequently transferred cultures would show an increase in growth rate. Six cultures (Carrot No 2 and 18; Lettuce No 7 and 13; Bean No 8 and 12) were grown in the dark on the reciprocal shaker on Murashige medium with no supplement (M) or with addition of one gram per liter of phytone (MP) or tryptone (MT), and transferred at intervals of one, two, three, four, five and six weeks for 28 weeks. A 10% (by volume) inoculum was used. All cultures were grown in the dark on the reciprocating shaker at 28°C. Dry weights were taken on duplicate 10 ml samples.

Fig. 4. Effect of age of inoculum on growth of plant cell suspension cultures on Murashige medium. Dark 28°C.

10% (v/v) inoculum from suspension culture.

Inoculum age △ one week, ▲ two weeks, ○–○ three weeks, ● four weeks, □ five weeks, ■ six weeks.

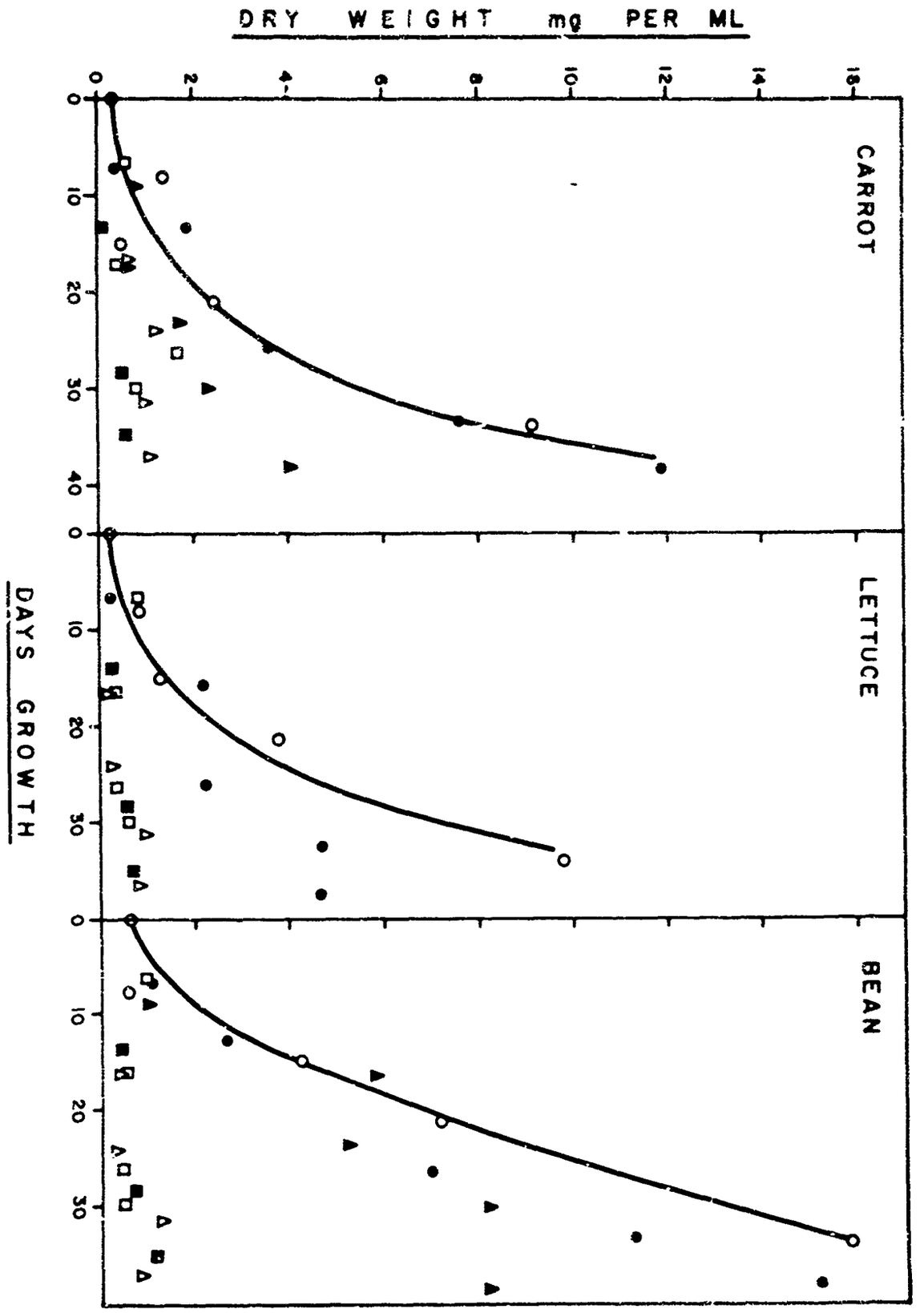

DRY WEIGHT mg PER ML
0
2
4
6
8
10
12
14
16
CARROT
LETTUCE
BEAN
DAYS GROWTH
0
10
20
30
40

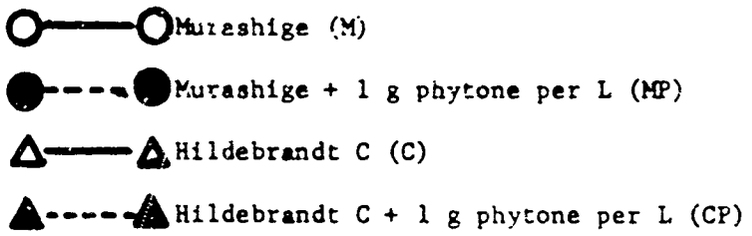
Figure 4

Fig. 5. Effect of medium on growth of plant cell suspension cultures.

Dark 28°C.

10% (v/v) inoculum from suspension culture on Murashige medium.

Inoculum age bean (2) - 2 weeks; bean (3), carrot, lettuce - 3 weeks.



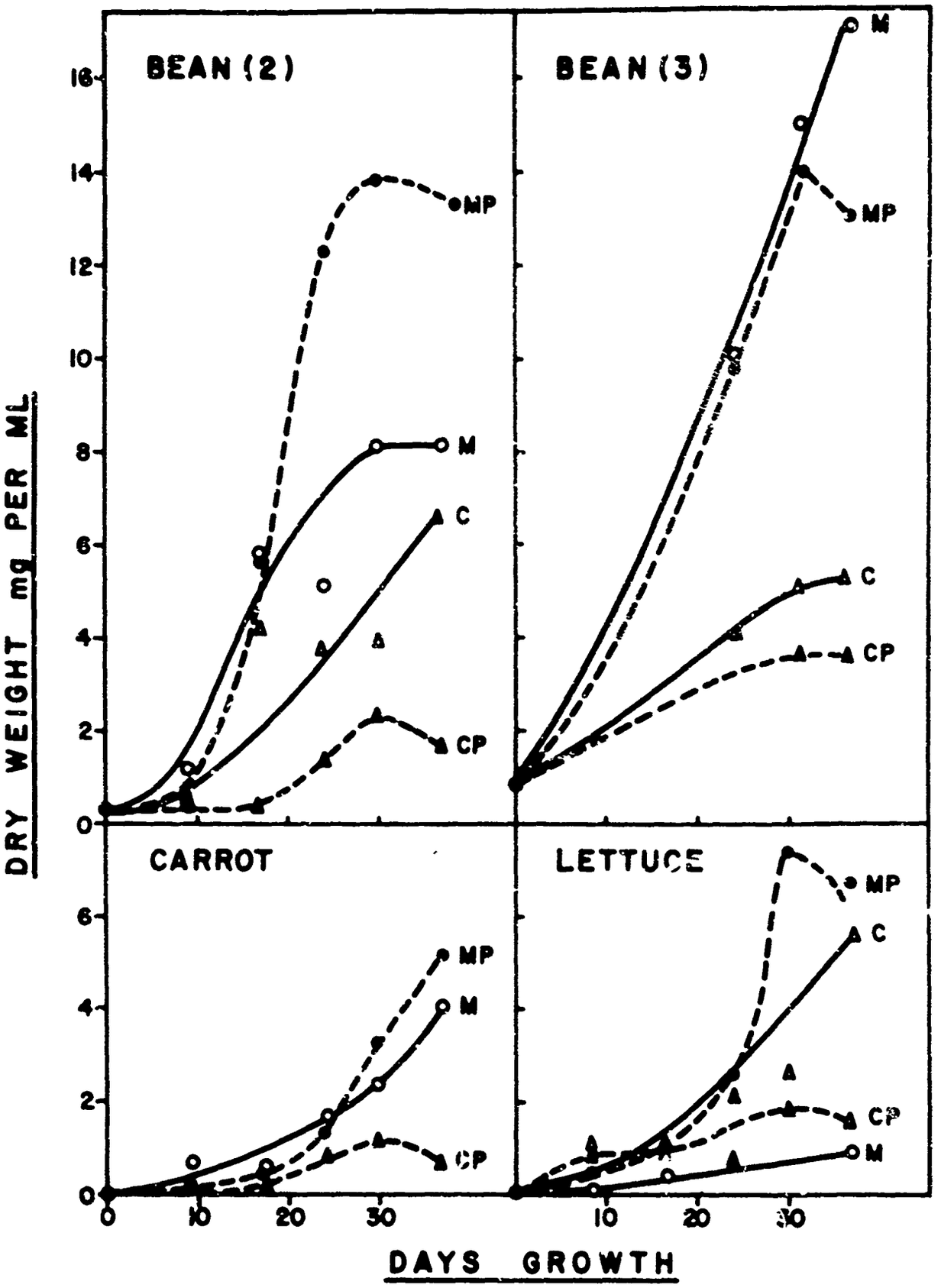

BEAN (2)
BEAN (3)
CARROT
LETTUCE
MP
M
C
CP
DRY WEIGHT mg PER ML
DAYS GROWTH
0
2
4
6
8
10
12
14
16
0
10
20
30

Figure 5 31

Although not all series were set up, and some were not completed, because of either slow growth rate or contamination, a great quantity of data were collected in this experiment. Selections from it are shown (Tables 10, 11, 12; Figures 6, 7, 8, 9, 10). All cultures were variable. A culture would increase in growth rate for a while and then without any apparent reason, a decline would occur. Or a poorly growing series would suddenly begin growing vigorously.

Growth increments usually did not exceed 15 per week when good inoculum was used. However, occasionally a culture with a very small inoculum would suddenly begin to grow, resulting in an abnormally high value for the growth increment. Such cultures never maintained this high growth increment in succeeding transfers. The extreme cases observed were:

Culture	Medium	Series	Inoculum	Final	GI	GI per week
8 bean	M	1 wk	.13mg/ml	11.2mg/ml	85.	85.
13 lettuce	M	2 wk	.13mg/ml	12.4mg/ml	94.	47.
18 carrot	MT	1 wk	.02mg/ml	.85mg/ml	41.	41.
18 carrot	MT	1 wk	.03mg/ml	1.9mg/ml	62.	62.
18 carrot	MT	2 wk	.04mg/ml	4.2mg/ml	104.	52.

Such values would not have occurred if inoculum had been standardized by weight instead of volume. These values do suggest that occasionally for brief periods exponential growth does occur in these plant cell cultures. Values for growth increment per week were highest in cultures transferred weekly and declined as the transfer interval lengthened (Figures 6, 7, 8, 9).

Table 10. Effect of Frequency of Transfer on Growth of Suspension Cultures of Carrot Cells Dark 28°C.

Transfer Interval	Medium →	Culture No. 2			Culture No. 18		
		M	MP	MT	M	MP	MT
1 week	No weeks	19	19	14	16	19	16
	Ave P	0.46	0.65	0.75	0.10	0.22	0.20
	Ave GI	10.1	11.2	10.4	9.6	10.0	14.6
	Ave final wt	3.6	5.1	5.8	0.59	2.3	1.6
2 weeks	No weeks	22	22	16	26	24	16
	Ave P	0.50	0.48	0.55	0.31	0.25	0.38
	Ave GI	6.2	6.5	6.2	8.8	9.5	12.8
	Ave final wt	7.5	7.5	8.3	4.9	3.7	6.0
3 weeks	No weeks	24	24	6	27	27	6
	Ave P	0.37	0.37	0.14	0.17	0.23	0.25
	Ave GI	4.1	3.6	2.6	5.7	7.1	7.2
	Ave final wt	8.5	8.5	3.4	4.0	5.2	5.3
4 weeks	No weeks	28	16	8	28	20	8
	Ave P	.19	.26	.23	.22	.25	0.40
	Ave GI	2.9	6.0	2.4	3.3	2.7	2.1
	Ave final wt	5.8	7.4	7.7	6.8	6.8	12.0
5 weeks	No weeks	20	10	-	10	10	-
	Ave P	0.21	0.18	-	0.16	0.21	-
	Ave GI	2.8	3.2	-	1.4	2.6	-
	Ave final wt	8.0	6.9	-	6.3	8.8	-
6 weeks	No weeks	18	12	-	18	6	-
	Ave P	0.19	0.20	-	0.20	0.14	-
	Ave GI	2.5	2.9	-	2.0	1.7	-
	Ave final wt	8.5	8.6	-	8.9	6.2	-

P = mg dry wt per ml per day

GI = growth increment per week

$$\frac{\text{final wt}}{\text{Inoculum}} - 1$$

M = Murashige Medium

MP = M + 1 g Phytone/L

MT = M + 1 g Tryptone/L

Table 11. Effect of Frequency of Transfer on Growth of Suspension Cultures of Lettuce Cells. Dark 28°C.

Transfer Interval	Medium →	Culture No. 7			Culture No. 13		
		M	MP	MT	M	MP	MT
2 weeks	No weeks	22	26	16	26	26	18
	Ave P	0.14	0.24	0.23	0.3[illegible]	0.38	0.47
	Ave GI	5.8	4.8	4.9	7.9	5.8	6.4
	Ave final wt	1.1	3.7	3.6	5.0	6.1	6.4
3 weeks	No weeks	27	24	6	27	27	6
	Ave P	0.13	0.15	0.12	0.18	0.31	0.26
	Ave GI	4.8	4.7	3.8	3.1	3.9	7.0
	Ave final wt	3.0	3.5	2.5	4.1	7.4	5.6
4 weeks	No weeks	28	20	8	28	20	8
	Ave P	0.07	0.19	0.24	0.22	0.25	0.40
	Ave GI	3.2	4.4	3.8	3.3	2.7	2.1
	Ave final wt	2.1	5.3	7.3	6.8	6.8	0.12
5 weeks	No weeks	15	20	10	20	15	-
	Ave P	0.12	0.24	0.27	0.25	0.26	-
	Ave GI	2.4	2.7	2.3	2.2	2.3	-
	Ave final wt	4.8	9.0	11.7	9.7	9.6	-
6 weeks	No weeks	18	12	-	18	12	-
	Ave P	.12	.28	-	.19	.31	-
	Ave GI	1.7	3.2	-	1.6	2.5	-
	Ave final wt	5.5	11.4	-	8.7	13.5	-

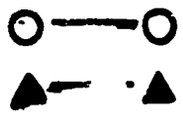
See Table 10

Table 12. Effect of Frequency of Transfer on Growth of Suspension Cultures of Bean Cells. Dark 28°C.

Transfer Interval	Medium →	Culture No. 8			Culture No. 12		
		M	MP	MT	M	MP	MT
1 week	No weeks	18	19	18	19	19	19
	Ave P	0.33	1.12	0.39	.45	.78	.81
	Ave GI	11.4	10.0	7.7	11.1	9.9	10.2
	Ave final wt	2.7	8.7	3.1	3.5	6.0	6.2
2 weeks	No weeks	26	26	20	26	26	18
	Ave P	0.66	0.75	0.83	0.44	0.67	0.74
	Ave GI	4.9	5.5	5.7	6.0	5.2	5.7
	Ave final wt	10.5	11.5	12.8	6.8	10.5	10.7
3 weeks	No weeks	27	15	6	27	21	6
	Ave P	0.52	0.38	0.61	0.35	0.25	0.39
	Ave GI	3.2	2.6	2.9	4.1	4.2	3.2
	Ave final wt	12.7	9.5	13.4	8.0	5.8	8.8
4 weeks	No weeks	28	16	8	28	16	4
	Ave P	0.45	0.39	0.45	0.20	0.09	0.01
	Ave GI	2.5	8.8	2.8	5.5	2.2	0.8
	Ave final wt	14.0	12.0	14.0	6.2	2.8	0.20
5 weeks	No weeks	20	10		15	10	
	Ave P	0.32	0.35		0.12	0.16	
	Ave GI	3.5	1.7		3.8	2.3	
	Ave final wt	13.3	14.0		4.6	6.0	
6 weeks	No weeks	18	6		18	12	
	Ave P	0.24	0.33		0.22	0.27	
	Ave GI	1.5	2.7		2.5	2.9	
	Ave final wt	11.1	14.0		10.1	11.1	

See Table 10

Fig. 6. Effect of frequency of transfer on growth rate of suspension cultures of plant cells over a 28 week period. Dark 28°C.



Murashige medium

Murashige + 1 g phytone per L

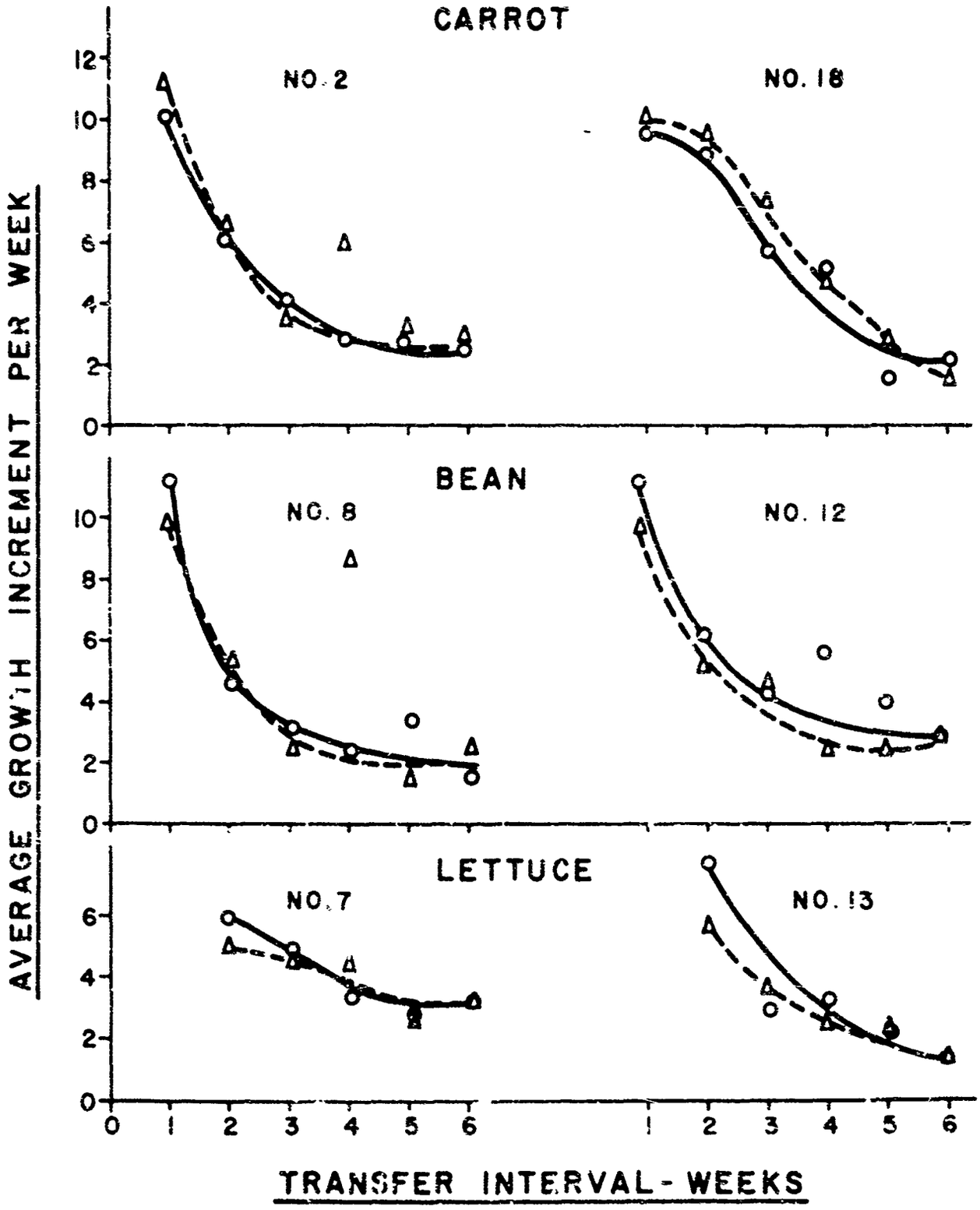
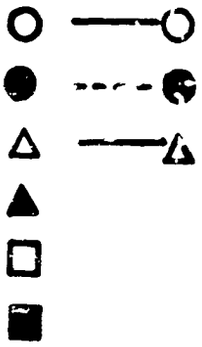

CARROT
NO. 2
NO. 18
BEAN
NO. 8
NO. 12
LETTUCE
NO. 7
NO. 13
AVERAGE GROWTH INCREMENT PER WEEK
TRANSFER INTERVAL - WEEKS

Figure 6

Fig. 7. Effect of frequency of transfer on cumulative growth increment of carrot cells in suspension culture on Murashige medium. Dark 28°C.



transfer every week

transfer every two weeks

transfer every three weeks

transfer every four weeks

transfer every five weeks

transfer every six weeks

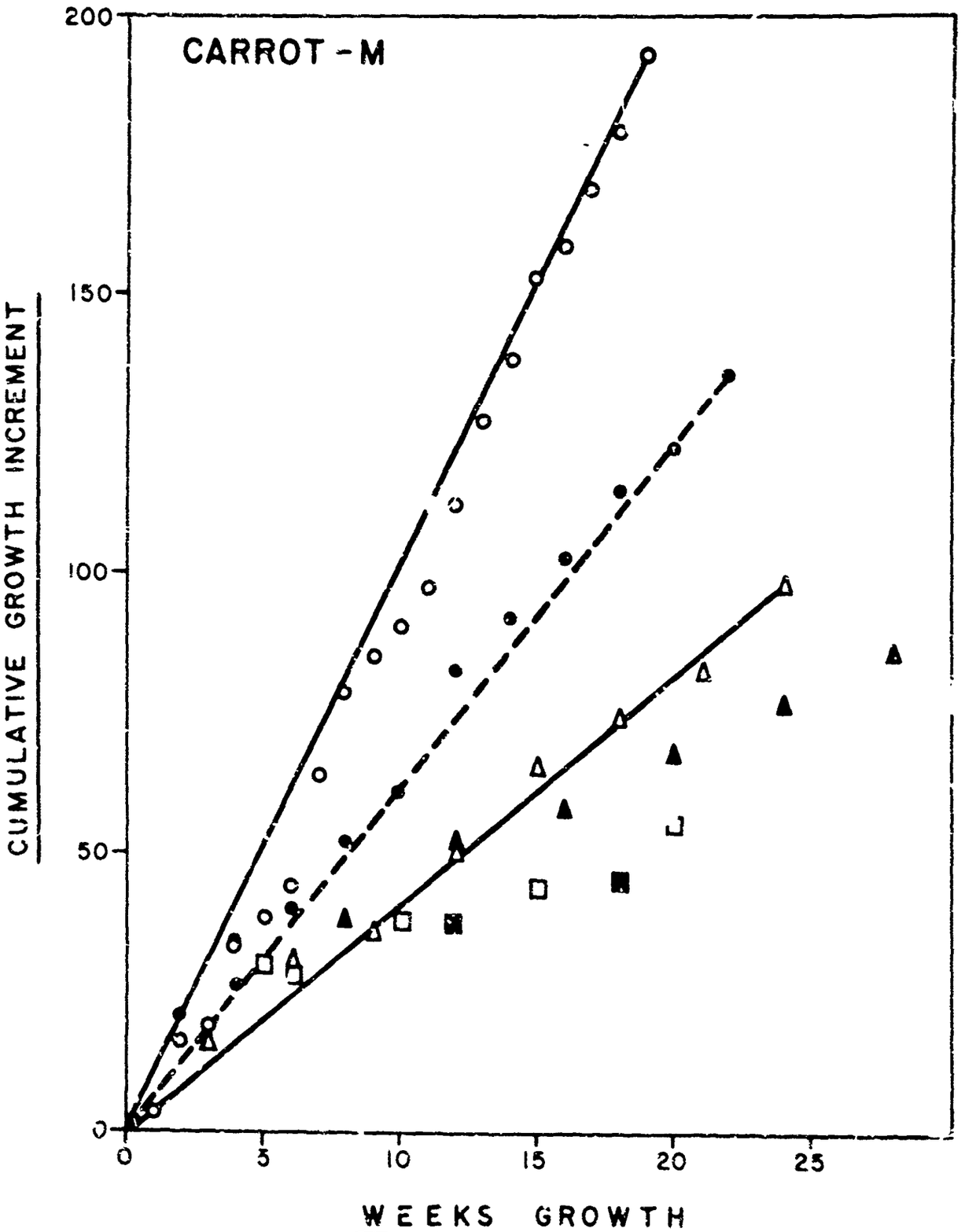
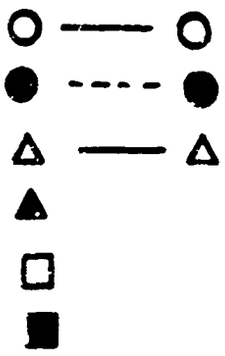

CARROT - M
CUMULATIVE GROWTH INCREMENT
200
150
100
50
0
0
5
10
15
20
25
WEEKS GROWTH

Figure 7

Fig. 8. Effect of frequency of transfer on cumulative growth increment of bean cells in suspension culture on Murashige medium. Dark 28°C.



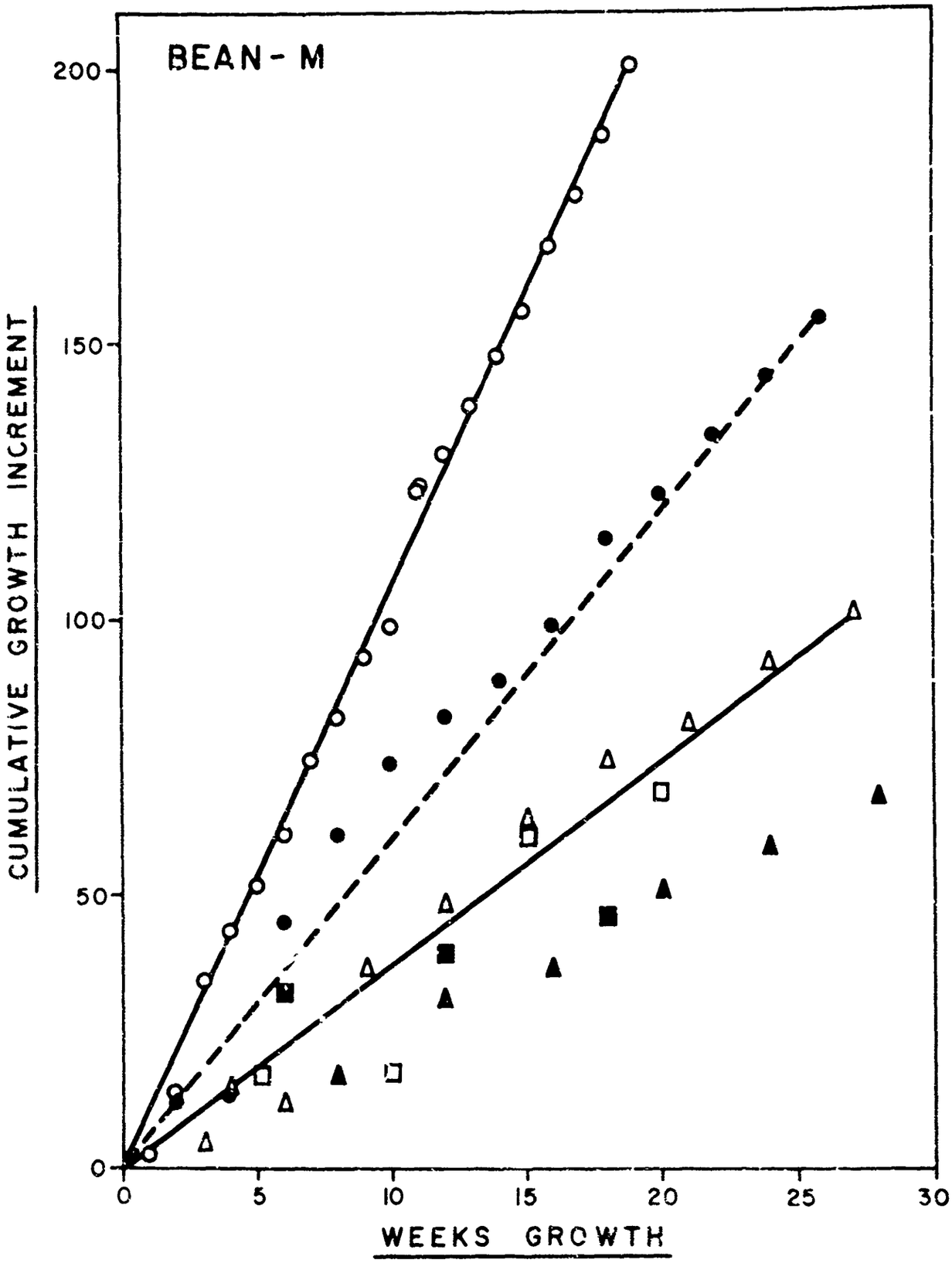
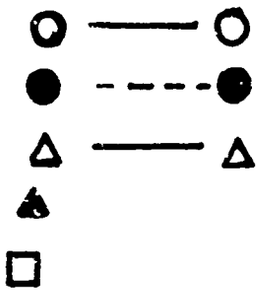

BEAN - M
200
150
100
50
0
0
5
10
15
20
25
30
CUMULATIVE GROWTH INCREMENT
WEEKS GROWTH

Figure 8 41

Fig. 9. Effect of frequency of transfer on cumulative growth increment of bean cells in suspension culture on Murashige medium supplemented with one gram of phytone per liter. Dark 28°C.



transfer every week

transfer every two weeks

transfer every three weeks

transfer every four weeks

transfer every five weeks

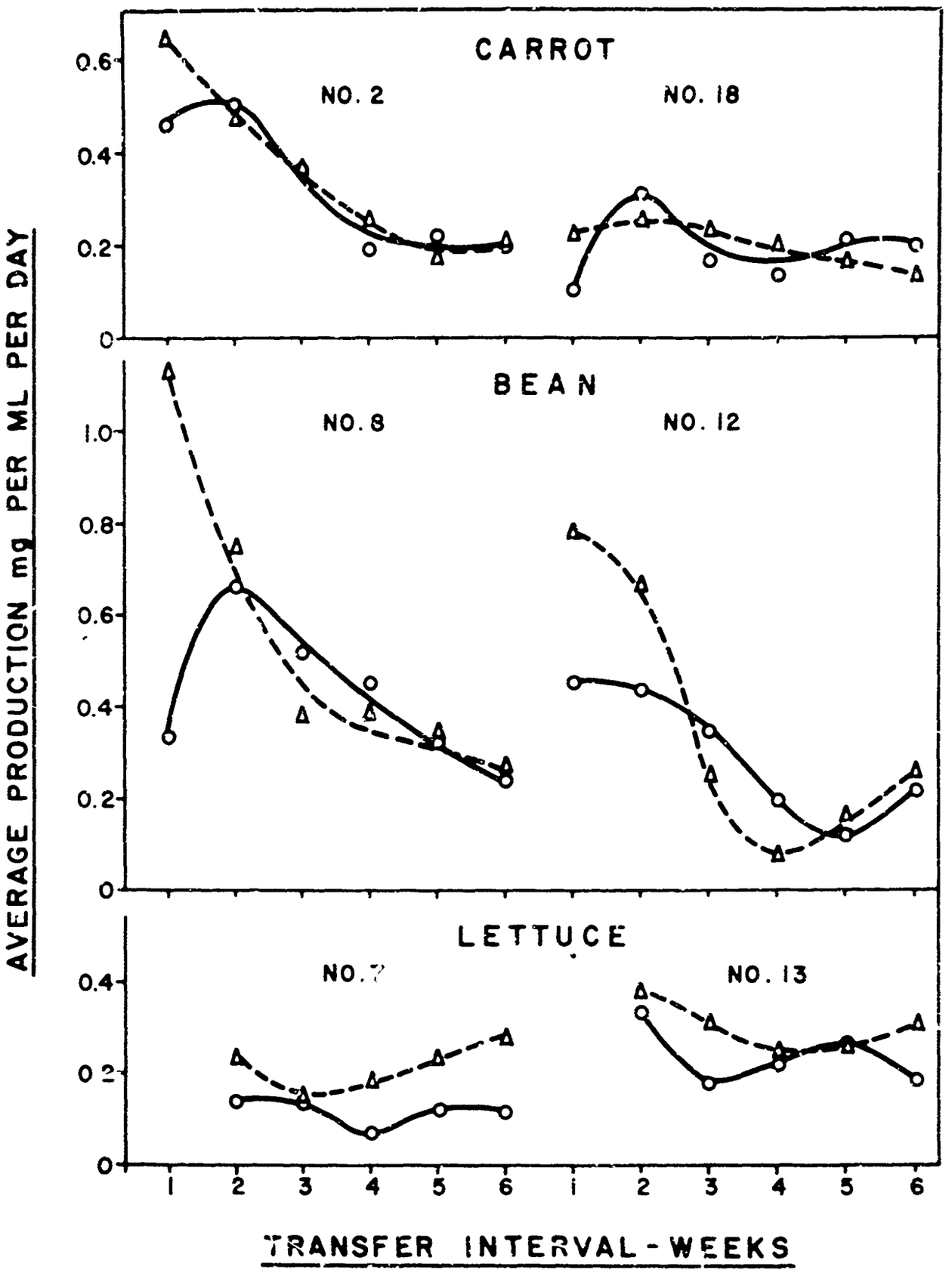

CARROT
NO. 2
NO. 18
BEAN
NO. 8
NO. 12
LETTUCE
NO. 7
NO. 13
AVERAGE PRODUCTION mg PER ML PER DAY
0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
TRANSFER INTERVAL - WEEKS

Figure 9

Fig. 10. Effect of frequency of transfer on productivity of dry matter in suspension cultures of plant cells over a 28 week period. Dark 28°C.

Murashige medium

Murashige + 1 g phytone per L

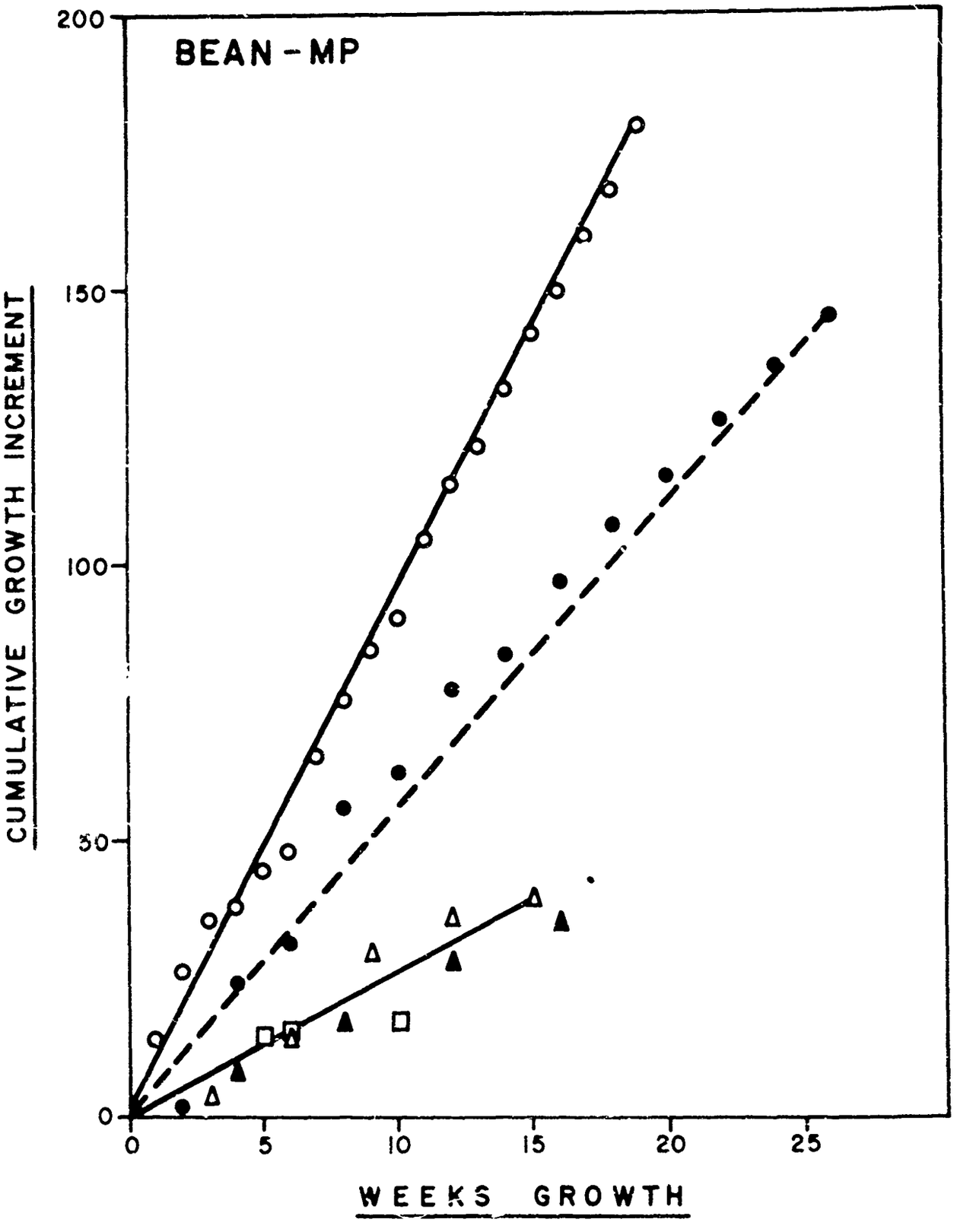

BEAN - MP
CUMULATIVE GROWTH INCREMENT
200
150
100
50
0
0
5
10
15
20
25
WEEKS GROWTH

Figure 10 45

Since our objective is production of cell mass the figures for dry weight produced in mg per ml per day are really more meaningful than growth increment. The best values obtained for P were:

Culture	Medium	Series	Transfer No.	P mg per ml per day
Bean	M	1 wk	19	1.65
Bean	MP	1 wk	4	1.70
Bean	MT	1 wk	13	1.91
Carrot	M	1 wk	19	1.14
Carrot	MP	1 wk	19	1.33
Carrot	MT	1 wk	3	1.09
Lettuce	M	2 wk	12	0.88
Lettuce	MP	2 wk	12	0.85
Lettuce	MT	2 wk	6	0.64

Average values for P were greatest on MP medium in the series transferred weekly. The best values on M medium usually occurred in the series transferred every other week (Figure 10). In any series, values for P fluctuated with successive transfer, but the long range trend for short intervals transfer was towards an increase in P value (data not shown). This suggests that we were having some success in selecting line of cells adapted to growth on the shaker. So far we have failed to stabilize such cultures.

Addition of phytone (hydrolyzed soy protein) is often favorable to young fast-growing cultures. In older or slower-growing cultures, soluble nitrogenous compounds to nourish meristematic cells are probably secreted by mature cells or released by broken cells.

Experiment 3. Effect of thickening agents.

One cause of our failure to obtain exponential growth in shake cultures could be that the large highly vacuolated thin walled plant cells were damaged by the shaking. In an attempt to prevent such damage we added various colloidal materials, mostly polysaccharides, at 4 g per liter to the medium (Table 13). All such media foamed excessively and were very slow to filter suggesting they would be difficult to handle under mass culture conditions. Therefore, in spite of some favorable effects, especially for lettuce cells, these agents have not been used again.

Table 13. Effect of Thickening Agents on Growth of Plant Cell Suspension Cultures Murashige Medium + 1g phytone per L - Dark 28°C. 28 days.

Additive	Carrot No 2	Lettuce No 7	Bean No 8	Bean No 12
0.4%	Dry Weight mg per ml			
None	7.9	1.6	8.5	6.6
Dextran	7.9	2.7	8.5	9.7
Carageenin	5.4	2.0	5.7	7.0
Guar Gum	6.0	4.9	9.8	8.4
Alginic acid	8.6	7.1	8.0	10.9
Gelatine	4.9	1.4	6.5	8.0
Pectin	10.4	0.8	4.3	9.3
CMC40	7.5	2.8	13.8	10.7
CMC70	8.8	5.4	8.4	7.4
HEC	5.7	4.4	0.6	8.0
Cellulose acetate	8.4	2.6	1.3	4.9
Methylcellulose	5.0	2.5	5.1	5.6
Cellulose SO_4	4.9	3.4	7.6	8.3
Carbowax	4.8	4.4	9.9	9.2
PVPK90	4.0	2.5	8.3	8.6
PVP189	5.1	2.3	7.8	6.2

CMC carboxymethyl cellulose
HEC hydroxyethyl cellulose
PVP polyvinylpyrillidone

Discussion

This study was carried out to investigate the growth of plant cells on simple media and the feasibility of growing such cells for food. We find that callus cultures can be readily isolated from many edible plants and maintained for well over a year on the fully defined medium of Murashige and Skoog. Growth of plant cell cultures after an initial lag is linear, rather than exponential as found for many microbial cells. Although exponential growth of cultured tobacco cells has been reported (5) this represented an increase in dry weight only from about 0.06 to 1.0 mg per ml. In our experiments inoculum weights were usually from 0.5 to 1.0 mg per ml and growth continued to 10 mg per ml or more. Similar growth curves are found for intact plants since cell division is restricted to a few meristematic regions.

We have used two indices of growth - growth increments per unit time, and productivity of dry matter in mg per ml per day. The growth increment, which is a multiplication factor, tends to be inversely proportional to inoculum size because of the linear growth curve. In static cultures (on solid media) maximum dry weights of up to 12.5 mg per ml of medium are attained. Sustained growth increments over a 36-week period averaged 0.2 - 1.5 per week. The higher figure was for bean cells transferred weekly. Productivity of dry matter ranged from 0.02 - 0.26 mg per ml per day. In suspension cultures (on liquid media) maximum dry weights of up to 23 mg per ml were obtained.

Sustained growth increments over a 28-week period ranged from 0.3 - 14.6 per week. Production of dry matter ranged from 0.07 - 1.12 mg per ml per day. The highest figure was for bean cells on medium supplemented with phytone and transferred weekly. There was some tendency for frequently transferred cultures to show an increasing growth rate, and for infrequently transferred cultures to decline in growth rate. It is commonly observed that stability is the exception in plant cell cultures. Growth rates, capability for differentiation, and nutrient requirements all vary (10). Our results include many examples to support this statement, but despite fluctuations the production of dry matter under controlled conditions does vary around a steady state. Yields of our fastest growing culture (bean) are compared with yields reported by other workers (Table 14). Is a sustained yield of one mg per ml per day good enough for profitable production? If not, how much can we hope to increase yields?

How do these values compare to yields of plant matter from conventional crops? Values of from one to eighty metric tons of dry organic matter per hectare per year have been reported for plants growing under optimum conditions (25) (Table 15). A metric ton is 1000 kg or 10^9 mg. A hectare is one hectometer $(100M)^2$ or 10^8 cm^2. Therefore, one metric ton per hectare equals 10 mg per cm^2 per year or 0.0275 mg per cm^2 per day, and 80 metric tons per hectare equals 2.2 mg per cm^2 per day. It

Table 14. Yields of Plant Cell Material in Suspension Culture.

Worker	Plant	Med.	Vessel	Mg per ml per day Wet	Dry	Reference
Nickell and Tulecke	Ginkgo, Lolium, Ilex	+ CM*	Carboy	3.		14
" "	Rose	+ CM*	Pilot Plant	10.		14
Byrne	Carrot	+ CM*	Carboy	6.1		2
"	"	"	Fermentor		1.9	3
Staba	Spearmint	Defined	Carboy	4.7		23
"	"	"	Flask	6.3	0.4	23
Tulecke	Rose	Defined	Phytostat	11.	0.4	21
Mandels	Bean	Defined	Flask		0.7	This paper
"	"	+ Phytone	"		1.1	

*Coconut milk 10-15%.

Table 15. Plant Yields in Total Dry Organic (Ash Free) Matter in Metric Tons per Hectare per Year. After Westlake 1963 (25).

Rainforests, perennials cultivated in tropics	50-80
Algae in sewage ponds + excess CO_2	45
Fertile reed swamps	30-45
Coniferous forests, cultivated perennials	25-40
Marine plants	21-40
Deciduous forests, cultivated annuals, uncultivated herbs	10-25
Fresh water macrophytes	13-21
Phytoplankton	1-9

is difficult to make a direct comparison between values based on volume and area, but it would appear that at present our yields in culture are of the same order of magnitude as crops grown conventionally under optimum conditions where year round agriculture is possible. However, the conventionally grown plant contains a high percentage of inedible waste. The cultured cell presumably will be entirely edible. Moreover, we hope that we can achieve a productivity of 10 mg/ml./day or greater, to give us a decided advantage over conventional agriculture.

REFERENCES

1. Becker, G. E., Hui, P., Albersheim, P. Synthesis of extracellular polysaccharide by suspensions of Acer pseudoplatanus cells. Plant Phys. 39, 913-920. 1964.

2. Byrne, A. and Koch, R. Food Production by submerged culture of plant tissue cells. Science 135, 215-216. 1962.

3. Byrne, A. Food Production by Tissue Culture Techniques, Interim Report. Armed Forces Food & Container Institute, AMXFC No. 2-63. 13 pp. 1963.

4. Carew, D. P., and Staba, E. J. Plant Tissue culture; its fundamentals, application, and relationship to medicinal plant studies. Lloydia 28, 1-26. 1965.

5. Filner, P. Studies on exponential cultures of plant cells. PhD Thesis. California Institute of Technology. 1965.

6. Gautheret, R. J. La culture des tissus vegetaux. Techniques et realizations, Masson et Cie Editeurs, Paris. 863 pp. 1959.

7. Hildebrandt, A. C. Tissue and single cell cultures of higher plants as a basic experimental method. In M. V. Tracey and H. F. Linskens (Ed) Modern Methoden der Pflanzenanalyse, Vol. V, p. 383-421. 1962. (Springer Verlag Berlin).

8. Hildebrandt, A. C., Wilmer, J. O., Johns, H. and Riker, A. J. Growth of edible chlorophyllous plant tissues in vitro. Am J. Bot. 248-254. 1963.

9. Laetsch, W. M. and Stetler, D. Chloroplast structure and function in cultured tobacco tissue. Am. J. Bot. 52, 798-804. 1965.

10. Murashige, T. and Nakano, R. Morphogenetic behavior in tobacco tissue cultures and implication of plant senescence. Am J. Bot. 52, 819-827. 1965.

11. Murashige, T. and Skoog, F. A revised medium for rapid growth and bio-assay with tobacco tissue cultures. Physiol. Plantarum 15, 473-497. 1962.

12. Nickell, L. J. The continuous submerged cultivation of plant tissue as single cells. Proc. Nat. Acad. Sciences 42, 848-850. 1956.

13. Nickell, L. J. Submerged growth of plant cells. Adv. Appl. Microbiol. 4, 213-236. 1962.

14. Nickell, L J. and Tulecke, W. Submerged growth of cells of higher plants. J. of Bioch. and Microbiol. Tech. and Eng. 11, 287-297. 1960.

15. Staba, E. J. Production of cardiac glycosides by plant tissue cultures I. Nutritional requirements in tissues of Digitalis lanata and Digitalis purpurea. J. Pharm. Sci. 51, 249-254. 1962.

16. Staba, E. J. The biosynthetic potential of plant tissue and cultures. Dev. in Industrial Microbiology 4, 193-198. 1963.

17. Stetler, D. A. and Laetsch, W. M. Kinetin induced chloroplast maturation in cultures of tobacco tissue. Science 149, 1387-1388. 1965.

18. Torrey, J. G. and Reinert, J. Suspension cultures of higher plant cells in synthetic media. Plant Phys. 36, 483-491. 1961.

19. Tulecke, W. Recent progress and the goals of plant tissue culture. Bull Torrey Bot. Club 88, 350-360. 1961.

20. Tulecke, W. Research on tissue cultures of higher plants. Air Force Report AMRL-TDR-63-124. 27 pp. 1963.

21. Tulecke, W. Growth of tissues of higher plants in continuous liquid culture and their use in a nutritional experiment. Air Force Report AMRL-TR-65-101. 37 pp. 1965.

22. Tulecke, W. and Nickell, L. Production of large amounts of plant tissue by submerged culture. Science 130, 863-864. 1959.

23. Wang, C. and Staba, E. J. Peppermint and Spearmint Tissue Culture. II. Dual carboy culture of spearmint tissues, Jour. Pharm. Sci. 52, 1058-1062. 1963.

24. Weinstein, L. H., Tulecke, W., Nickell, L. G. and Laurencot, H. J. Biochemical and physiological studies of tissue cultures and the plant parts from which they are derived. Contr. Boyce Thompson Inst. 21, 371-386. 1962.

25. Westlake, D. F. Comparisons of plant productivity. Biol. Rev. 38, 385-425. 1963.

26. White, P. R. The cultivation of animal and plant cells. Ronald Press., N. Y. 246 pp. 2nd Ed. 1963.

27. Wimber, D. E. Clonal multiplication of Cymbidiums through tissue culture of the shoot meristem. Amer. Orchid Soc. Bull. 32, 105-107. 1963.

28. Van Overbeek, J., Conklin, M. and Blakeslee, A. F. Cultivation in vitro of small Datura embryos. Am. J. Bot. 29, 472-477. 1942.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
U. S. Army Natick Laboratories Natick, Massachusetts	None
	2b. GROUP

3 REPORT TITLE

GROWTH OF PLANT CELL CULTURES
I. Isolation of Cultures, Selection of Media, and Effects of Frequency of Transfer

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (First name, middle initial, last name)

Mary Mandels, Anne Maguire and Hamed M. El-Bisi

6 REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO OF REFS
July 1967	56	28

8a. CONTRACT OR GRANT NO	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1J014501A71C	68-6-FL
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) FL-58
d.	

10 DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11 SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U S. Army Natick Laboratories Natick, Massachusetts 01760

13 ABSTRACT

Callus structures have been isolated from a number of edible plants and maintained on simple defined media for extended periods. Growth rates are slow in comparison to other microbial systems, and increase tends to be linear. Static cultures on solid media double in 5-10 days, yield up to 0.26 mg dry weight per ml per day, and attain a maximum weight of about 12 mg dry weight per ml. Suspension cultures double in 2-5 days, yield up to 1.1 mg dry weight per ml per day, and attain a maximum weight of about 23 mg dry weight per ml. These growth rates are of the same order of magnitude as those for higher plants growing conventionally. Considerable improvements in these growth rates will be required before use of plant cell cultures as food can be realized economically.

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified

Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Growth	3		7			
Plant cells	8,9		7			
Culture media	9		6			
Food	4					
Personnel	4					
Transferring			6			
Productivity			7			